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Essentiality, Expression, and Characterization of the Class II 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase of *Staphylococcus aureus*

E. IMOGEN WILDING,[1] DONG-YUL KIM,[2] ALEXANDER P. BRYANT,[1] MICHAEL N. GWYNN,[1]* R. DWAYNE LUNSFORD,[1] DAMIEN MCDEVITT,[1] JOSEPH E. MYERS, JR.,[1] MARTIN ROSENBERG,[1] DANIEL SYLVESTER,[1] CYNTHIA V. STAUFFACHER,[3] AND VICTOR W. RODWELL[2]

Department of Microbiology, SmithKline Beecham Pharmaceuticals, Collegeville, Pennsylvania 19426,[1] and Departments of Biochemistry[2] and Biological Sciences,[3] Purdue University, West Lafayette, Indiana 47907

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Sequence comparisons have implied the presence of genes encoding enzymes of the mevalonate pathway for isopentenyl diphosphate biosynthesis in the gram-positive pathogen *Staphylococcus aureus*. In this study we showed through genetic disruption experiments that *mvaA*, which encodes a putative class II 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, is essential for in vitro growth of *S. aureus*. Supplementation of media with mevalonate permitted isolation of an auxotrophic *mvaA* null mutant that was attenuated for virulence in a murine hematogenous pyelonephritis infection model. The *mvaA* gene was cloned from *S. aureus* DNA and expressed with an N-terminal His tag in *Escherichia coli*. The encoded protein was affinity purified to apparent homogeneity and was shown to be a class II HMG-CoA reductase, the first class II eubacterial biosynthetic enzyme isolated. Unlike most other HMG-CoA reductases, the *S. aureus* enzyme exhibits dual coenzyme specificity for NADP(H) and NAD(H), but NADP(H) was the preferred coenzyme. Kinetic parameters were determined for all substrates for all four catalyzed reactions using either NADP(H) or NAD(H). In all instances optimal activity using NAD(H) occurred at a pH one to two units more acidic than that using NADP(H). pH profiles suggested that His378 and Lys263, the apparent cognates of the active-site histidine and lysine of *Pseudomonas mevalonii* HMG-CoA reductase, function in catalysis and that the general catalytic mechanism is valid for the *S. aureus* enzyme. Fluvastatin inhibited competitively with HMG-CoA, with a K_i of 320 μM, over 10^4 higher than that for a class I HMG-CoA reductase. Bacterial class II HMG-CoA reductases thus are potential targets for antibacterial agents directed against multidrug-resistant gram-positive cocci.

Isoprenoids, which are ubiquitous in nature, comprise a family of over 23,000 products, each composed of repeating five-carbon, isopentenyl diphosphate (IPP) subunits. The principal products of IPP in bacteria include the lipid carrier undecaprenol, which is involved in cell wall biosynthesis (33), menaquinones and ubiquinones involved in electron transport (29), and carotenoids (19). Two pathways for the biosynthesis of IPP have been described, the mevalonate pathway (16) and the glyceraldehyde-3-phosphate (GAP)-pyruvate pathway (36, 37). Analysis of the distribution of the genes encoding enzymes involved in the two pathways revealed that *Bacillus subtilis* and many gram-negative bacteria, including *Escherichia coli*, *Haemophilus influenzae*, and *Helicobacter pylori*, possess only genes that encode the GAP-pyruvate pathway, while the low-G+C gram-positive cocci and *Borrelia burgdorferi* possess only genes that encode the mevalonate pathway (41). However, the functionality of the implied polypeptides of the mevalonate pathway in bacteria has not been demonstrated.

Enzymes involved in the mevalonate pathway in a number of organisms, including humans, have been isolated and studied. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) is the best-characterized and rate-limiting enzyme of the pathway (9) and is the target of the statin class of cholesterol-lowering drugs (1). Based on amino acid sequence analysis, Bochar et al. (8) suggested that there are two distinct classes of HMG-CoA reductase. Genes that appear to encode class I HMG-CoA reductases are present in all eukaryotes, in many archaea, and in some streptomycetes. By contrast, genes that encode class II forms of the enzyme are present in some eubacteria and in the archaeon *Archaeoglobus fulgidus* (41). Previously characterized class I HMG-CoA reductases include those of the archaea *Haloferax volcanii* (6) and *Sulfolobus solfataricus* (7, 22), the gram-positive eubacterium *Streptomyces* sp. strain CL190 (40), and numerous eukaryotes. Characterized class II HMG-CoA reductases include that from *A. fulgidus* (23) and the biodegradative *Pseudomonas mevalonii* enzyme (4, 20), whose structure has been determined (24, 39) and which converts mevalonate to HMG-CoA, permitting growth on mevalonate (15). No biosynthetic eubacterial class II HMG-CoA reductase has, however, been characterized.

Biosynthetic HMG-CoA reductases catalyze reaction 1, the reductive deacylation of the thioester group of HMG-CoA to the primary alcohol of mevalonate using 2 mol of NADPH (35). The putative intermediates, mevaldyl-CoA and mevaldehyde, remain bound during the course of the reaction. HMG-CoA reductases also catalyze three additional reactions. Reactions 2 and 3 of free mevaldehyde appear to model the second reductive stage and the reverse of the first reductive stage of reaction 1, respectively. Reaction 4, the oxidative acylation of (*R*)-mevalonate to (*S*)-HMG-CoA, is the reverse of reaction 1.

$$(S)\text{-HMG-CoA} + 2\,\text{NADPH} + 2\,\text{H}^+ \rightarrow (R)\text{-mevalonate} + \text{CoASH} + 2\,\text{NADP}^+ \quad (1)$$

$$(R)\text{-mevaldehyde} + \text{NADPH} + \text{H}^+ \rightarrow (R)\text{-mevalonate} + \text{NADP}^+ \quad (2)$$

* Corresponding author. Mailing address: Department of Microbiology, SmithKline Beecham Pharmaceuticals, 1250 South College Rd., Collegeville, PA 19426. Phone: (610) 917-7749. Fax: (610) 917-4989. E-mail: Mick_Gwynn-1@sbphrd.com.

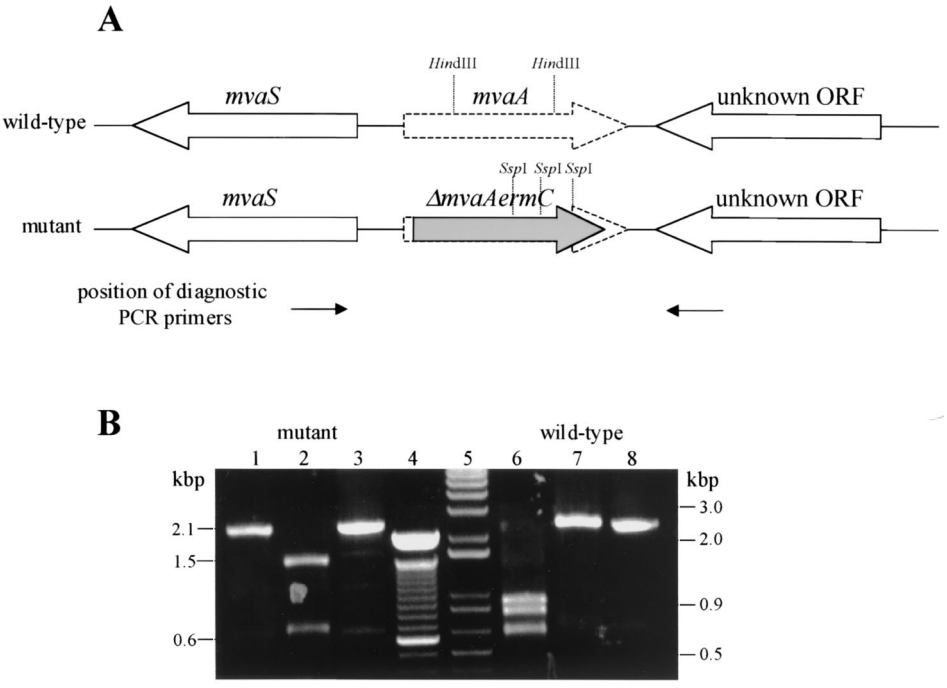

FIG. 1. Chromosomal analysis of the *S. aureus mvaA* null mutant. (A) The chromosome of the *mvaA* null mutant contains an allelic replacement of *mvaA* (dotted block arrow) with the *ermC* gene (gray block arrow). PCR primers (small arrows) were designed to anneal to regions flanking *mvaA*. (B) Products of the expected size were amplified using chromosomal DNA from the mutant (2,288 bp [lane 3]) or wild type (2,371 bp [lane 8]). Replacement of *mvaA* with *ermC* resulted in the elimination of two *Hin*dIII sites and the introduction of three *Ssp*I sites in the chromosome. Amplified fragments were digested with either *Hin*dIII or *Ssp*I, and products of the expected size were obtained. Lane 1, the mutant PCR product incubated with *Hin*dIII (undigested); lane 2, the mutant PCR product cut with *Ssp*I (1,518 and 659 bp and two smaller bands); lane 6, wild-type PCR product cut with *Hin*dIII (894, 776, and 618 bp); lane 7, wild-type PCR product incubated with *Ssp*I (undigested); lanes 4 and 5, 100-bp and 1-kb DNA markers, respectively.

$$(R)\text{-mevaldehyde} + \text{NADP}^+ + \text{CoASH} \rightarrow (S)\text{-HMG-CoA} + \text{NADPH} + \text{H}^+ \quad (3)$$

$$(R)\text{-mevalonate} + 2\,\text{NADP}^+ + \text{CoASH} \rightarrow (S)\text{-HMG-CoA} + 2\,\text{NADPH} + 2\,\text{H}^+ \quad (4)$$

where CoASH is reduced coenzyme A.

We report here that the *mvaA* gene of the gram-positive pathogen *Staphylococcus aureus* is essential for growth, and we report the cloning, purification, and characterization of the *mvaA* gene product of *S. aureus*, the first truly biosynthetic class II HMG-CoA reductase characterized from a eubacterium.

MATERIALS AND METHODS

Reagents. Purchased reagents included restriction enzymes, calf intestinal alkaline phosphatase, T4 DNA ligase, protein and DNA molecular weight markers (Gibco BRL Life Technologies), *Pfu* Turbo DNA polymerase (Stratagene), and lysostaphin (Applied Microbiology, Inc.). Fluvastatin was a gift from Novartis. Unless otherwise specified, all other chemicals were from Sigma.

Plasmid, bacterial strains, and culture media. Expression plasmid pET28 was from Novagen. Bacterial strains used included *E. coli* BL21(DE3) and DH5α and *S. aureus* strains RN4220 (31), WCUH29 (NCIMB 40771), and NCTC 8325-4. Luria-Bertani broth and agar (38) served for growth of *E. coli*, and tryptone soy broth (TSB) and tryptone soy agar (TSA) (Oxoid) served for growth of *S. aureus*. Where required, the *E. coli* medium was supplemented with 50 μg of kanamycin per ml and 1% (wt/vol) glucose and the *S. aureus* medium was supplemented with 0.5 μg of erythromycin and 5 μg of tetracycline per ml.

DNA techniques. Plasmid DNA was isolated using the RPM kit (Bio 101 Inc.) or the Wizard Midiprep DNA purification system (Promega). PCR products were isolated by horizontal agarose gel electrophoresis and purified using the GENECLEAN II kit (Bio 101 Inc.). Chromosomal DNA was isolated from *S. aureus* using standard procedures (27). Incubation with 0.1 μg of lysostaphin per ml was included during the preparation of plasmid and chromosomal DNA from *S. aureus* to facilitate cell lysis. Procedures for DNA restriction, dephosphorylation and ligation, agarose gel electrophoresis, PCR, transformation of $CaCl_2$-competent *E. coli*, and phage transduction were performed as described by Sambrook et al. (38) or as recommended in the manufacturers' instructions. PCR employed a RoboCycler gradient temperature cycler (Stratagene). Synthetic oligonucleotides were prepared, and automated DNA sequencing was performed in-house at SmithKline Beecham.

Determination of essentiality of the *mvaA* gene. A DNA construct for allelic replacement of the *S. aureus mvaA* gene was generated by an overlap three-piece PCR technique. This technique is a gene fusion procedure (42) extended to include two separate long flanking sequences (547 bp directly upstream and 591 bp directly downstream of the *mvaA* gene) surrounding a central selectable cassette containing the 1,234-bp *ermC* gene from pE194 (17). The final full-length products were digested and cloned into the *Bam*HI site of a pBluescript II KS(+) derivative containing the tetracycline resistance gene (*tetK*) from pT181 (21). The resulting plasmids were introduced into *S. aureus* RN4220 by electroporation with selection for resistance to erythromycin. All of the colonies isolated, which were also resistant to tetracycline (cointegrants), were examined for target-specific plasmid integration by diagnostic PCR with primer pairs based on plasmid- and locus-specific sequences. Bona fide plasmid cointegrants were transferred back to *S. aureus* RN4220 and NCTC 8325-4 by phage φ11 transduction to allow for a second recombination event that could potentially resolve to generate an allelic-replacement mutation. Transductants resistant to erythromycin and sensitive to tetracycline were examined for allelic replacement by PCR.

Murine hematogenous pyelonephritis infection model. Cells from overnight cultures of *S. aureus* NCTC 8325-4 and the *mvaA* null mutant grown in TSB with 10 mM mevalonate as required were centrifuged, washed twice in sterile phosphate-buffered saline, and adjusted to an A_{600} of 0.2. Female CD-1 mice weighing 18 to 20 g (Charles River, Quebec, Canada) were inoculated by tail vein injection with 0.2 ml of a suspension containing approximately 10^7 bacteria. Mice were monitored twice daily for signs of illness. All animals were euthanized by carbon dioxide overdose 5 days after inoculation. Both kidneys were removed using an aseptic technique and homogenized together in 1 ml of PBS, and the number of viable bacteria was determined by plating on TSA, which was supplemented with 10 mM mevalonate for the *mvaA* null mutant.

Construction of the HMG-CoA reductase expression plasmid. The *mvaA* gene was amplified from *S. aureus* WCUH29 chromosomal DNA using primers which introduced *Nhe*I and *Bam*HI sites at the ends of the amplified fragment. The 1,278-bp PCR fragment was cloned using the pCR-Zero Blunt cloning kit (Invitrogen) and, using the introduced restriction sites, was subcloned into pET28 in frame with the N-terminal histidine tag and thrombin cleavage site to form pJO1. The insert was sequenced to verify that no errors had been introduced during PCR amplification.

Expression and purification of HMG-CoA reductase. *E. coli* BL21(DE3) cells containing pJO1 were grown to mid-log phase, induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and harvested 2.5 h postinduction. To confirm expression, total cell lysates were prepared as outlined by Sambrook

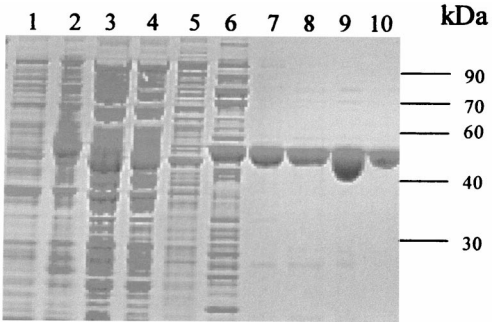

FIG. 2. Expression of *S. aureus mvaA* and purification of the gene product. A protein of the anticipated size was absent from induced *E. coli* BL21(DE3) cells containing the parent vector (lane 1) but was expressed in cells containing pJO1 (lane 2). Lane 3, crude cytosol; lane 4, material that failed to bind to the nickel column. Bound protein was eluted with imidazole at concentrations of 10 mM (lane 5), 50 mM (lane 6), 100 mM (lane 7), and 500 mM (lane 8). Lanes 9 and 10 contain 20 and 4 μg of purified protein, respectively.

et al. (38) and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Purification of HMG-CoA reductase employed a 500-ml culture of *E. coli* BL21(DE3) transformed with pJO1. Following induction, the cells were harvested by centrifugation and HMG-CoA reductase was purified using the HisTrap kit (Amersham Pharmacia Biotech) essentially as described in the manufacturer's instructions, but with the following modifications. Cell lysis was performed by addition of 0.2 mg of lysozyme per ml and incubation for 20 min at 37°C. Lysates were frozen in a dry ice-ethanol bath, thawed, and sonicated three times for 10 s, with 10 s between bursts. The freezing-and-sonication procedure was repeated four times. Protein was eluted from the column with 10, 50, 100, and 500 mM imidazole. Purified HMG-CoA reductase in a solution of 50 mM KH_2PO_4 (pH 7.5), 200 mM NaCl, 1 mM dithiothreitol (DTT), 1 mM EDTA, and 50% (wt/vol) glycerol was stored at −20°C. Protein concentrations were determined by using the Bradford assay (10) using bovine serum albumin as a standard.

Assays of HMG-CoA reductase activities. Spectrophotometric assays of HMG-CoA reductase activity employed a Hewlett-Packard model 8452 diode array spectrophotometer whose cell compartment was maintained at 37°C during measurements at 340 nm of the oxidation or reduction of NAD(P)H. Assays were conducted in a final volume of 200 μl. Standard assay conditions for each reaction studied were as follows.

Reaction 1, reductive deacylation of HMG-CoA to mevalonate. Assays using NADPH as the coenzyme contained 0.25 mM NADPH, 0.25 mM (*R*,*S*)-HMG-CoA, 50 mM NaCl, 1 mM EDTA, 5 mM DTT, and 25 mM KH_2PO_4 (pH 7.5). Assays using NADH as the coenzyme contained 0.25 mM NADH, 0.25 mM (*R*,*S*)-HMG-CoA, 50 mM NaCl, 1 mM EDTA, 5 mM DTT, and 25 mM KH_2PO_4 (pH 6.0).

Reaction 2, reduction of mevaldehyde to mevalonate. Assays contained 0.5 mM NADPH, 8.0 mM (*R*,*S*)-mevaldehyde, 50 mM NaCl, 1 mM EDTA, 5 mM DTT, and 25 mM KH_2PO_4 (pH 7.0).

Reaction 3, oxidative acylation of mevaldehyde to HMG-CoA. Assays contained 0.5 mM $NADP^+$, 5.0 mM coenzyme A, 8.0 mM (*R*,*S*)-mevaldehyde, 50 mM NaCl, 1 mM EDTA, 5 mM DTT, and 100 mM Tris-HCl (pH 9.0).

Reaction 4, oxidative acylation of mevalonate to HMG-CoA. Assays contained 5 mM $NADP^+$, 5.0 mM coenzyme A, 6.0 mM (*R*,*S*)-mevalonate, 50 mM NaCl, 1 mM EDTA, 5 mM DTT, and 100 mM Tris-HCl (pH 9.0).

Unless otherwise stated, all reactions were initiated by adding HMG-CoA, mevaldehyde, or mevalonate. For all assays, one enzyme unit represents the turnover, in 1 min, of 1 μmol of nicotinamide nucleotide coenzyme. This corresponds to the turnover of 1 μmol of mevaldehyde or to 0.5 μmol of HMG-CoA or mevalonate. Reported data are mean values for at least triplicate determinations.

RESULTS

Essentiality of the *mvaA* gene in *S. aureus*. The genes involved in the synthesis of IPP via the mevalonate pathway are essential for in vitro growth in *Streptococcus pneumoniae* (41). Therefore, it was of interest to determine whether *S. pneumoniae* is representative of the gram-positive cocci and if HMG-CoA reductase is essential in other pathogens. Attempts were made to delete *mvaA* from the chromosome of *S. aureus* using an allelic-replacement mutagenesis strategy. For nonessential genes the cointegrant can be readily resolved using phage transduction to isolate the mutant of interest at a frequency of 0.5 to 5%. In this case, however, it proved impossible to resolve the cointegrant structure and isolate a mutant on standard growth media, strongly suggesting that *mvaA* is essential for in vitro growth.

When the growth media were supplemented with mevalonate, *mvaA* auxotrophic mutants were readily obtained and the allelic replacement was confirmed using diagnostic PCR and restriction analysis. Oligonucleotide primers designed to

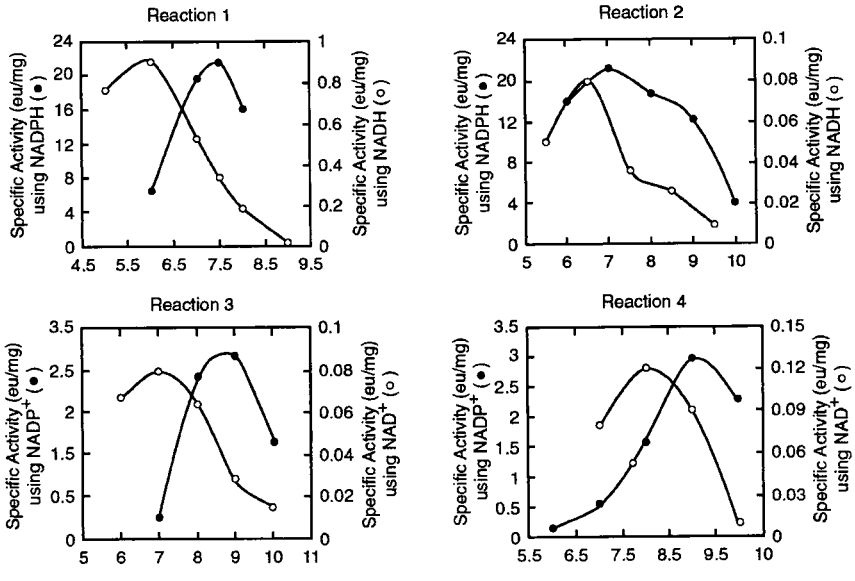

FIG. 3. Effect of hydrogen ion concentration on activity. The effects of the indicated concentrations of hydrogen ion on the rates of the indicated reactions were assayed under standard conditions other than pH using either NADP(H) (●) or NAD(H) (○) as the coenzyme. Assays were conducted in a solution of 50 mM KCl, 25 mM K-PO_4, and 100 mM Tris-HCl adjusted to the indicated pH values (*x* axis). (Top left) Reaction 1, reductive deacylation of HMG-CoA to mevalonate; (top right) reaction 2, reduction of mevaldehyde to mevalonate; (lower left) reaction 3, oxidative acylation of mevaldehyde to HMG-CoA; (lower right) reaction 4, oxidative acylation of mevalonate to HMG-CoA. eu, enzyme units.

TABLE 1. Comparison of K_m values for the class II *S. aureus* HMG-CoA reductase with those of the class II biodegradative enzyme from *P. mevalonii* and the class I biosynthetic hamster enzyme[a]

Reaction and substrates	K_m (μM) of enzyme from:		
	S. aureus	*P. mevalonii*	Hamster
Reaction 1 (HMG-CoA→mevalonate)			
HMG-CoA	40	20	20
NADPH	70		80
NADH	100	80	
Reaction 2 (mevaldehyde→mevalonate)			
Mevaldehyde	6,900	8,000	1,600
NADPH	240		160
Reaction 3 (mevaldehyde→HMG-CoA)			
Mevaldehyde	570	80	90
CoASH	490	110	50
$NADP^+$	40		600
Reaction 4 (mevalonate→HMG-CoA)			
Mevalonate	670	260	20
CoASH	390	60	10
$NADP^+$	580		510

[a] Data for *P. mevalonii* and hamster HMG-CoA reductase are from reference 34.

anneal either to regions flanking *mvaA* (Fig. 1) or to internal regions of *mvaA* or *ermC* (data not shown) were used to amplify chromosomal DNA templates prepared from the wild-type and mutant strains. The expected products were obtained, confirming that *mvaA* had been replaced by *ermC* in the auxotrophic mutant.

Auxotrophy of *mvaA* null mutants. The minimal concentration of mevalonate required for the growth of the *S. aureus* *mvaA* null mutant was investigated in TSA containing mevalonate at 0.01, 0.1, and 1 mM and in TSA without supplementation. Addition of mevalonate to the medium enabled the mutant to grow, although the rate of growth was lower at lower concentrations of mevalonate. In the absence of added mevalonate, the mutant showed no growth after 60 h on rich medium (TSA).

The minimum concentration of mevalonate required by the *mvaA* null mutant for overnight growth in TSB was 1 mM (data not shown). The effect of removing mevalonate from the medium on the viability of *mvaA* null mutants was investigated. An overnight culture of the *mvaA* null mutant grown in the presence of 1 mM mevalonate was diluted 10^6-fold into fresh media with and without mevalonate. The number of viable cells was determined by plating in the presence of 1 mM mevalonate. In the presence of mevalonate the mutant grew well, and the viable count increased by 4 logs over 24 h. When the mevalonate concentration was reduced to 1 nM, however, the *mvaA* null mutant was unable to grow, although viability was unaffected (data not shown).

TABLE 2. Relative abilities of three class II HMG-CoA reductases to use NADP(H) and NAD(H)

Source of HMG-CoA reductase	$k_{cat(NADPH)}/k_{cat(NADH)}$ for[a]:		Reference
	Reaction 1	Reaction 4	
S. aureus	25	25	This study
A. fulgidus	0.5	0.05	23
P. mevalonii		4.4×10^{-4}	14

[a] k_{cat}, turnover number.

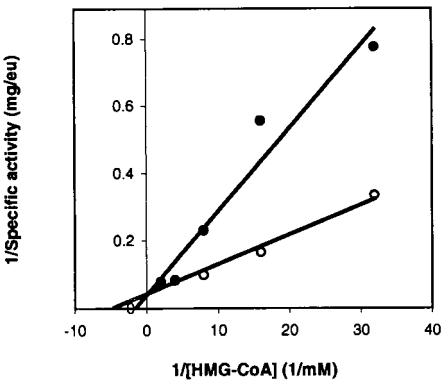

FIG. 4. Inhibition by a statin drug. Double-reciprocal plot for inhibition of reaction 1, the reductive deacylation of HMG-CoA to mevalonate, using NADPH as the coenzyme. Analyses were conducted at pH 7.5 and 37°C at the indicated concentrations of HMG-CoA in the presence of 0 μM (○) or 500 μM (●) fluvastatin. All reactions were initiated by adding NADPH. eu, enzyme units.

Virulence attenuation of the *mvaA* null mutant. The *mvaA* mutation was transferred to the pathogenic strain *S. aureus* NCTC 8325-4 by phage ϕ11 transduction. The resulting mutants were auxotrophic for mevalonate. Five mice were inoculated intravenously with either *S. aureus* NCTC 8325-4 or the *mvaA* null mutant and sacrificed 5 days postinfection (none of the mice died during the infection). Viable *S. aureus* NCTC 8325-4 harboring the deletion could not be recovered from the kidneys (i.e., the level was below the limit of detection of 1.6 $\log_{10}$ CFU/mouse) when plated in the presence of mevalonate, in contrast to the wild type, *S. aureus* NCTC 8325-4, which was present at 5.17 ± 0.17 $\log_{10}$ CFU/mouse (mean ± standard deviation for five mice). The results indicate that the auxotrophic *mvaA* null mutant has greatly reduced virulence in the hematogenous pyelonephritis infection model.

Expression of *mvaA* and purification of *S. aureus* HMG-CoA reductase. The *mvaA* gene of *S. aureus* was cloned into pET28 in frame with the N-terminal histidine tag and thrombin cleavage site. A soluble protein was expressed in *E. coli* BL21(DE3) to high levels following induction with 1 mM IPTG and was readily purified on a nickel-chelating column, the majority eluting in the 500 mM imidazole fraction (Fig. 2). Typical yields were 30 mg of over 90% homogenous protein per liter of induced culture. The *mvaA* gene encodes a putative protein of 425 residues with a calculated molecular weight of 46,180. Matrix-assisted laser desorption ionization mass spectrometry analysis indicated that the purified protein had a molecular weight of 48,503, which corresponds to the full-length HMG-CoA reductase fusion protein minus the terminal methionine residue. N-terminal sequencing of the purified protein confirmed that this residue had been removed and that the N-terminal sequence was otherwise intact.

Biochemical characterization. The enzymatic activity of the purified protein was determined by measuring the oxidation or reduction of NAD(P)H at 340 nm. Firstly, the temperature dependency of *S. aureus* HMG-CoA reductase for the deacylation of HMG-CoA was investigated. Optimal activity for the catalysis of reaction 1 was observed between 35 and 45°C and decreased precipitously above 45°C (data not shown). All subsequent assays were therefore conducted at 37°C.

Coenzyme specificity of *S. aureus* HMG-CoA reductase was

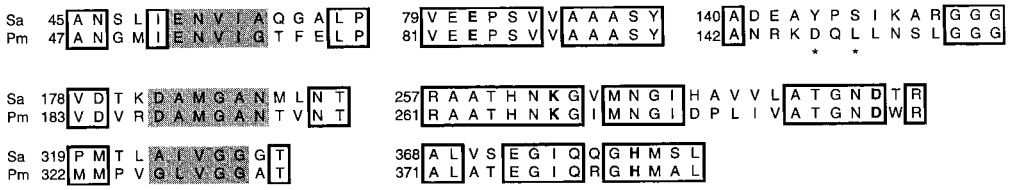

FIG. 5. Alignment of selected amino acid sequences of the class II HMG-CoA reductases from *S. aureus* (Sa) and *P. mevalonii* (Pm). Conserved paired residues $E81_S$-$E83_P$, $K263_S$-$K267_P$, $D279_S$-$D283_P$, and $H378_S$-$H381_P$, all in bold, represent active-site residues known to function in catalysis by the *P. mevalonii* enzyme. The conserved ENVIG, DAMGXN, and GTVGG sequences are shaded. $Asp146_P$ and $Leu148_P$, marked with asterisks, contribute to the ability of *P. mevalonii* HMG-CoA reductase to discriminate against NADP(H). Additional residues that are conserved in both sequences are boxed. The sequences of *S. aureus* and *P. mevalonii* HMG-CoA reductases are 39% identical overall. Alignment was performed using the MegAlign sequence analysis software from DNASTAR Inc., Madison, Wis.

investigated over a range of pH values. Figure 3 illustrates the pH profiles for catalysis of reactions 1 to 4 using either NADP(H) or NAD(H) as the coenzyme. Unlike most other characterized HMG-CoA reductases, the *S. aureus* enzyme can use both NADP(H) and NAD(H) as coenzymes for catalysis of all four reactions. While significant activity was observed using NAD(H), NADP(H) was in all instances the preferred coenzyme. For all four reactions, optimal activity using NAD(H) occurred at a pH one to two units more acidic than that using NADP(H).

K_m values were determined for all substrates for the four reactions catalyzed by HMG-CoA reductases. Table 1 summarizes these data and includes a comparison to the K_m values for the class II HMG-CoA reductase of *P. mevalonii* and to the class I Syrian hamster enzyme. In Table 2 the coenzyme specificities of the three characterized class II HMG-CoA reductases are compared.

The effect of the statin drug fluvastatin on *S. aureus* HMG-CoA reductase was investigated. Statin drugs are competitive inhibitors of HMG-CoA reductase activity (9), and as expected, catalysis of reaction 1 was inhibited by fluvastatin. Inhibition was competitive with respect to HMG-CoA, with a K_i of 320 μM (Fig. 4).

DISCUSSION

We demonstrated that the *mvaA* gene is essential for the growth of *S. aureus* and that *mvaA* null mutants could be readily isolated on media supplemented with mevalonate. These results support previous observations that *mvaA* is essential in *S. pneumoniae* (41) and strongly suggest that the gene is essential in other gram-positive cocci which use the mevalonate pathway for the synthesis of IPP, as the GAP-pyruvate pathway has not been found in these organisms. The *S. aureus mvaA* null mutant was severely attenuated in the mouse hematogenous pyelonephritis infection model. Although the plasma mevalonate concentration in mice has not been measured, the concentrations in humans and rats, 0.02 to 0.08 μM and 0.08 to 0.50 μM, respectively (32), are well below that required to support the growth of the *mvaA* null mutant.

Derived sequence similarities and conservation of known active-site residues and motifs (Fig. 5) suggested that the *mvaA* gene of *S. aureus* encodes a class II HMG-CoA reductase (41). Expression in *E. coli* of an *mvaA*-polyhistidine tag construct and the subsequent purification and enzymatic characterization of the gene product revealed that this was indeed the case. Inspection of the pH profiles for catalysis of all four reactions revealed that two functional groups with pK_a values around 7 and 9 must be in their protonated states for activity. We propose that these are His378 of *S. aureus* ($His378_S$) and $Lys263_S$ (Fig. 5), the apparent cognates of active-site residues His381 of *P. mevalonii* ($His381_P$) and $Lys267_P$ of *P. mevalonii* HMG-CoA reductase that have established functions in catalysis (9). We further infer that the mechanism proposed for catalysis by *P. mevalonii* HMG-CoA reductase (39) is valid for *S. aureus* HMG-CoA reductase.

All three characterized class II HMG-CoA reductases use NAD(H) (Table 2), but only the *S. aureus* and *A. fulgidus* enzymes use either NAD(P)H or NAD(H). In this respect, the *S. aureus* enzyme more closely resembles the archaeal enzyme than its mesophilic, biodegradative counterpart. The ability of *S. aureus* HMG-CoA reductase to use either coenzyme was predicted, since $Asp146_P$ and $Leu148_P$ are major determinants of nucleotide coenzyme specificity for *P. mevalonii* HMG-CoA reductase (14). Crystal structures of the *P. mevalonii* enzyme revealed that $Asp146_P$ interacts with the 2′-hydroxyl of the adenosyl ribose of NAD(H), discriminating against NADP(H) (24, 39). Consistent with the ability to use NADP(H), sequence alignments revealed no clear cognates of $Asp146_P$ or $Leu148_P$ in *S. aureus* HMG-CoA reductase. Despite significant similarity (39% identity and 57% similarity), the ability of *S. aureus* HMG-CoA reductase to accommodate both coenzymes suggests that significant differences in the coenzyme binding site may become apparent when the crystal structure of *S. aureus* HMG-CoA reductase is determined and compared to that of the *P. mevalonii* enzyme. Finally, we suggest that the ability to use both coenzymes may turn out to be a general property of biosynthetic class II forms of the enzyme.

Despite its ability to use NAD(H), *S. aureus* HMG-CoA reductase is a true biosynthetic enzyme. This is inferred from its preference for NADPH and the presence in *S. aureus* of genes that encode a putative HMG-CoA synthase, mevalonate kinase, phosphomevalonate kinase, and mevalonate decarboxylase (41). While most oxidoreductases exhibit a high order of coenzyme specificity, oxidoreductases that use either NADP(H) or NAD(H) include L-glutamate dehydrogenase (11, 12, 28), isocitrate dehydrogenase (13, 25), glucose-6-phosphate dehydrogenase (3, 5), 6-phosphogluconate dehydrogenase (5), aldose reductase (30), and biliverdin IXα reductase (26). With the exception of liver alcohol dehydrogenase (2), coenzyme K_m values are 1 or more orders of magnitude lower for NADP(H) than for NAD(H), suggesting a preference for NADPH. The HMG-CoA reductases of *A. fulgidus* and *S. aureus* thus are among a mere handful of oxidoreductases, and the only HMG-CoA reductases, for which the K_m is essentially the same for either coenzyme.

The K_i for inhibition of *S. aureus* HMG-CoA reductase by a statin drug is over 4 orders of magnitude higher than the K_i for a class I eukaryotic enzyme, suggesting that the two classes can be discriminated chemically. The recent publication of the crystal structure of the catalytic portion of the human HMG-CoA reductase (18) confirms large differences in the architecture of the active sites of the human and *P. mevalonii* enzymes.

Thus, agents selective for the inhibition of the class II bacterial enzymes might be achievable. The essential nature of this enzyme suggests that the class II HMG-CoA reductases represent a promising target for antibacterial agents directed against multidrug-resistant gram-positive cocci.

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